IV. Remarks and Conclusion

Claims 32-35 and 64-67 are currently pending. Claims 1-31 and 36-63 have been cancelled without prejudice or disclaimer to pursue the Claims of Invention II, as defined by the Examiner. The cancellations made were not based on reasons related to patentability under 35 U.S.C. §§ USC 101, 102, 103 and/or 112. No estoppel should result from said cancellations. Applicants expressly reserve the right to pursue the non-elected subject matter in a divisional application.

Applicants have amended Claim 64 to further claim a protein vaccine of Claim 32. Such amendment finds support on page 10, 5th paragraph of the specification. The amended Claims are in the spirit of the restriction requirement and will create no undue burden on the Examiner, as the Examiner has made the search.

In line item 5, the Examiner objects to the application as being informal for not having a Brief Description of the Figures and for the recitation of Claims on page 41. Applicants have amended the Claims to We Claim. Likewise, Applicants have amended page 37 to add "Brief Description of the Figures" as a new line. Accordingly, Applicants respectfully request reconsideration of the objections.

To cure the rejection under 35 USC §101, Applicants have amended Claim 32 to add the word "isolated" before the claimed protein. Support for the amendment can be found at page 19, Example 1.

Claims 32-35 and 34 stand rejected under 35 USC §112, 1st ¶, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the art that the inventors had possession of the claimed invention. The Examiner contends that the Applicants only disclose a recombinant B. canis protein having 15kD molecular weight and comprising the amino acid sequence as set forth in SEQ ID NO:2. The Examiner asserts that the specification does not disclose a B. canis protein having a molecular

weight 15kD and comprising an amino acid sequence that is at least 80%, 85%, 90% or 95% homologous to the amino acid sequence as depicted in SEQ ID NO: 2 or an immunogenic fragment thereof; or, a vaccine for combating a B. canis infection comprising a B. canis protein having 15kD molecular weight and comprising the amino acid sequence as set forth in SEQ ID NO:2. The Examiner asserts that the specification does not disclose a B. canis protein having a molecular weight 15kD and comprising an amino acid sequence that is at least 80%, 85%, 90% or 95% homologous to the amino acid sequence as depicted in SEQ ID NO: 2 or an immunogenic fragment thereof.

The Examiner asserts that Applicants' failure to disclose any substitution, insertion, or deletion or change (i) in the protein of SEQ ID NO: 2 to obtain 80%, 85%, 90% or 95% homologous to the amino acid sequence as depicted in SEQ ID NO: 2 or an immunogenic fragment thereof, or, (ii) a vaccine comprising said variants and another virus or microorganism pathogenic to dogs. Applicants respectfully request reconsideration in regards to the knowledge of one of ordinary skill in the art.

One of ordinary skill in the art recognizes that:

1. Protein variants may exist in nature with up to 20 % amino acid differences, while still constituting the same protein with respect to immunogenic characteristics, so 80% homology is only fair to cover natural variants of the protein of the claimed invention.
2. The comparison to Burgess et al. is not justified.
3. Only Claims directed towards immunogenic proteins are made. Such Claims are enabled. There is no reason for us to enable that which we are not claiming.

In further explanation of point 1, in the specification there is a section explaining the concept of variant proteins, having (conserved) differences in the amino acid sequence, but still constituting the same protein; see page 7 line 27 through page 8, l. 19. Several substitutions that "...do not essentially alter biological and immunological activities..." (specification, page 7, ll. 31-32) are specified. Also it is noted: "Such amino acid substitutions of the exemplary embodiments of the invention, as well as variations having deletions and/or insertions are within the scope of the invention as long as the resulting proteins retain immune reactivity." (specification, page 8, ll. 7-10). Consequently, proteins having 80 % or more homology to SEQ ID NO: 2 are only variants of the protein of the invention that are known to exist in nature. It would be unfair to deny an inventor rights to such known natural variants of the molecule of his invention.

In further explanation of point 2, the example from Burgess, on acidic fibroblast growth factor (AFGF) being inactivated upon a single amino acid mutation has no relation to the invention. Proteins of the invention are not such proteins that need to perform a biological function; on the contrary, they need to be immunologically active, i.e. antigenic, so that it can be recognized by the cells and molecules of the immune system of the target animal.

To be antigenic, a protein need not be biologically active. A protein that has lost biological activity, for example, as a result of denaturation or mutation can very well still function as antigen.

As an example of this concept, a comparison to inactivated vaccines illustrates the concept. The protein in such examples may be denatured through chemical or physical inactivation, but it is still recognized by the host's immune system.

The concept is also described in the specification in Example 2, on page 29-30 and Figures 8 and 10: results are presented from studies with the two proteins of the invention using such exemplary techniques as immunoprecipitation, immunoblotting and immunofluorescence. Each of these techniques (deliberately) employs chemicals and conditions that are known to denature proteins. Still the proteins are recognized by antibodies, allowing their detection and characterization. Accordingly, biological activity is of no relevance to antigenicity of proteins.

Similar to tolerance for denaturation, in respect of the antigenicity of a protein, there also is a wide tolerance to amino acid mutations and substitutions. Therefore, although a mutation or substitution of one or more amino acids in a protein may disturb its biological activity (as in Burgess), that mutation or substitution need not destroy its immunogenic regions, so called epitopes.

Proteins contain many such epitopes, which trigger an array of immune responses. Such epitopes can be 'linear', comprising a stretch of 4 or more amino acids, or 'conformational', comprising a spatial structure of molecular charges (see specification, page 4, lines 19-28). Either type of epitope presents a certain molecular structure to the immune system of the target animal.

A mutation or substitution could interrupt a particular linear epitope, but would leave the many other linear epitopes in tact. Even more so, a mutation or substitution in a conformational epitope would not suffer at all, as for instance a conservative amino acid substitution leaves the spatial structure of charges, and therefore the conformational epitope in tact.

Accordingly, amino acid changes are of little relevance for the antigenicity of a protein. Therefore, proteins differing as much as 20 % in their amino acid sequence may still constitute effectively the same antigenic protein. Although the examiner is correct in that a protein's

biological activity may be disturbed even by a single change in the amino acid sequence, this is not at all the case for a protein's antigenic characteristics.

It is for that reason that upon disclosure of an immunogenic protein, and a vaccine thereof, or a vaccine from a nucleic acid encoding such a protein, the immunogenicity of the variants of that protein is implicitly disclosed. Applicants respectfully request reconsideration.

In further explanation of point 3, the specification provides ample illustration of what is immunogenic: page 9, l. 16 through page 10, l. 9. Routine methods, well-known in the art for determining whether a fragment still contains immunogenic determinants (epitopes), are disclosed. Such methods employ experimental techniques, such as PEPSCAN, or (even more convenient) computer predictions. Either way, the skilled person is perfectly able to determine a protein's immunogenicity, without undue burden.

Consequently the immunogenic fragments as claimed are enabled by the description. The specification was written to disclose only that which we claim, immunogenic proteins and variants. Accordingly, whenever a variant protein is not an immunogenic protein according to the invention, this need not be enabled, as it is not claimed.

- In summary, the examiner admits the application is enabling for Bcvir15 and Bcvir32 proteins, as well as for their vaccines and diagnostics. Therefore, natural variants of these proteins are within 20 % homology and are fully disclosed in the specification also. Lastly, it is routine to determine the immunogenicity of such proteins and variants as disclosed in the specification. Therefore, immunogenic proteins, vaccines and diagnostics as claimed, of Bcvir15 and Bcvir32, as well as their protein variants to within 20 % homology are sufficiently disclosed in the application as filed.

As for the objections to Claims 65-67 as claiming incorrect dependency, Applicants respectfully request reconsideration. Applicants' attorney corrected the dependency on November 17, 2003.

Claims 32-35 and 64-67 stand rejected under 35 USC §112, 2nd ¶, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regards as the invention.

To begin, Applicants would agree with the Examiner that the conditions under which the molecular weight of a protein is determined are relevant to the measurement. In fact, such knowledge is common in the art. As is common knowledge in the art, the determination of the molecular weight of a protein by gel-electrophoresis is accurate to within +/- 5 %. Therefore, the Bcvir15 protein of the invention is not exactly 15 kDa, it is 15 kDa ± 5%. Applicants have amended Claim 32 accordingly. Support for said amendment may be found from the 15kD band in figures 7, 8, 9, 10, and 12. The band is not sharp, thereby illustrating the variation of such methods up to approximately 5%.

Applicants have amended Claim 66 to change "derived" to "obtainable." Support this broadening amendment is in the specification, pages 4-5. The concept of expression of the nucleic acid of the invention is described, thereby disclosing that the Bcvir15 protein of the invention is obtainable from a variety of sources.

With respect to the question on isolation from a virus or a microorganism, Applicants submit that all apply: the nucleic acid encoding the protein was originally isolated from Babesia canis (Example 1), which is a parasitic organism, but subsequently in a non-limiting example, the nucleic acid was expressed in Escherichia coli (Example 2) which is a bacterium. Similarly it may be expressed and obtained from a viral expression system, as described on page 11, l. 2-3 of the specification. Accordingly, Applicants respectfully request reconsideration.

Claims 32-35 and 64 stand rejected under 35 USC §102(b) as being anticipated by the 1998 article to Kulakov (hereinafter referred to as the Kulakov article). The Examiner asserts that the Kulakov article discloses that cell lysates from various Brucella species comprise 90-16kD antigens. The Examiner then asserts that Applicants' invention is inherent in the kulakov article, as a vaccine would be an intended use of the B. canis protein. Applicants respectfully request reconsideration of the rejection in light of this response.

There cannot be anticipation by the Kulakov article. The Kulakov article discloses proteins from Brucella canis, which is also abbreviated as B. canis, but of course Brucella is a genus of bacteria, which are single cell prokaryotes; whereas Babesia is an Apicomplexan parasitic organism, therefore a multicellular eukaryote.

Kulakow only discusses Brucella antigens (see title), therefore Kulakov is not relevant prior art, and does not anticipate the proteins of the invention.

This ignores the fact that from the Kulakov article abstract provided, one cannot verify the Examiners' statement, that Kulakov does actually discloses such an extract, let alone that the mentioning in an abstract from an article in Russian, of an extract spanning proteins in a range as broad as 16-98 kDa (undoubtedly containing a plethora of bands), could hardly be considered an

enabling disclosure for any one specific protein; whether that protein be of about 15 kDa in size or of another size. Accordingly, Applicants respectfully request reconsideration.

Claims 32-35 and 64-65 stand rejected under 35 USC §102(b) as being anticipated by the 1992 Schetters article (hereinafter referred to as the Schectters article. The Examiner asserts that the Schetters article discloses a vaccine comprising a B. canis associated protein in a supernatant. The Examiners contends that SEQ ID NO: 2 of Applicants' invention is an inherent property of the disclosed Schetters article supernatant. Applicants respectfully request reconsideration in light of this response.

Applicants are very familiar with this disclosure, as the assignee of the instant application and of the patent that originated from the teachings of the Schetter article are one in the same, Akzo Nobel. The proteins in the supernatant described in the Schetter article are known as exoantigens and are the subject of US patent 6,045,806.

To differentiate the Bcvir15 and Bcvir32 proteins of the invention from these exoantigens, immunoprecipitation experiments were performed and outlined in Example 2, the results of which are presented in Figure 8.

In brief: rabbit polyclonal antibodies were produced (p. 26) directed against E. coli expressed proteins of Bcvir15 (ORF 1) or Bcvir32 (ORF 2) (p. 25). Next, radioimmunoprecipitation assays of 35S labeled parasite cultures were performed (p. 27).

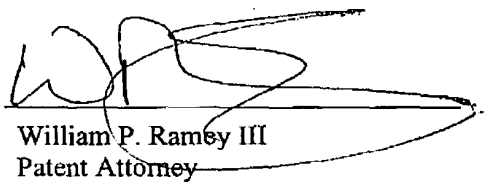
Results are described on pages 29-30 of the specification, and presented in Figure 8: in lanes 3, of Figure 8A and 8B, fractions of labeled antigens from the Babesia culture were incubated with a specific antibody. As is clear from these results, a band of about 15 kDa was

specifically precipitated only in the total antigen fraction of panel A, but not in the exoantigen fraction of panel B. This is also described in the specification, on p. 29, ll. 23-27.

The exoantigens described by Schetters et al., are not recognized by an antiserum specific for the Bcvir15 protein of the invention. Therefore Bcvir15 is not an exoantigen, and is not similar to the proteins in US 6,045,806. Accordingly, Applicants respectfully request reconsideration.

In conclusion, Applicants believe the Claims in a condition for allowance. Applicants respectfully request that the Examiner contact Applicants' attorney with any questions. Please charge any required fees and credit any credits to deposit account 02-2334.

Respectfully Submitted,



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